Effects of Topical Antiglaucoma Eye Drops on Prostaglandin E_2–Induced Aqueous Flare Elevation in Pigmented Rabbits

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Purpose. To evaluate the role of topical instillation of some antiglaucoma agents on experimental elevation of aqueous flare induced by prostaglandin E_2 (PGE_2) in pigmented rabbits.

Methods. Transcorneal diffusion of PGE_2 (25 μg/mL or 7.09×10^{-2} mM) with the use of a glass cylinder was achieved to produce aqueous flare elevation in pigmented rabbits. An antiglaucoma agent was topically administered before application of PGE_2. Aqueous flare was measured with a laser flare cell meter.

Results. A single instillation of apraclonidine 1.15%, two instillations of epinephrine 1.25%, two instillations of dipivefrin 0.1%, and two instillations and one instillation of dipivefrin 0.04% eye drops inhibited 98%, 96%, 87%, 73%, and 47% of PGE_2-induced aqueous flare elevation, respectively. Timolol 0.5%, nipradilol 0.25%, dorzolamide 1%, and pilocarpine 2% eye drops had no effects on the increase of PGE_2-induced flare.

Conclusions. Apraclonidine, epinephrine, and dipivefrin eye drops inhibit PGE_2-induced elevation of aqueous flare in pigmented rabbits. (*Invest Ophthalmol Vis Sci.* 2002;43: 1142–1145)

Studies from our laboratory have shown that transcorneal diffusion of prostaglandin E_2 (PGE_2) with use of a glass cylinder induces elevation of aqueous flare in pigmented rabbits and that the elevation is reproducible when PGE_2 is reapplied more than 1 week later.[1,2] A glass cylinder (11 mm in diameter) was attached to the cornea to avoid transconjunctival diffusion.[1]

Aqueous flare elevation decreased after repeated applications of PGE_2 within a short time (hourly or daily). However, weekly applications of PGE_2 did not change the aqueous flare reaction in pigmented rabbits.[2] We have also reported that a single instillation (30 minutes before PGE_2) of 0.25% clonidine and two instillations (60 and 30 minutes before PGE_2) of 0.5% betaxolol inhibits 89% and 32% of PGE_2-induced aqueous flare elevation, respectively.[3,4] In the present study, we evaluated the effect of topical instillation of antiglaucoma agents on aqueous flare elevation induced by PGE_2 in pigmented rabbits.

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Materials and Methods

Animals

Fifty-two pigmented male rabbits (Japanese mongrel) weighing 2.5 to 3.5 kg were used. The animals were housed and treated according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The study was approved by the Institutional Animal Care and Utilization Committee, Toyama Medical and Pharmaceutical University, Toyama, Japan. One eye of each animal was used to determine the effect of each drug. The eyes received two transcorneal applications of PGE_2 at 1- or 2-week intervals. Three months later, the other eye of the animal was used to determine the effect of another drug.

Chemicals

Dipivefrin hydrochloride (Pivalephrine, a β2-agonist) and pilocarpine (Sanpilo, a cholinergic agent) ophthalmic solutions were obtained from Santen Pharmaceutical Company (Osaka, Japan). Timolol maleate (Timoptol, a β-antagonist) and dorzolamide (Trusopt, a carbonic anhydrase inhibitor) were from Banyu Pharmaceutical Company (Tokyo, Japan). Apraclonidine (Iopidine, an α2-agonist), epinephrine (Epista, a β2-agonist), and nipradilol (Nipranol, a β-antagonist with an α1-antagonist) ophthalmic solutions were purchased from Alcon Laboratories (Fort Worth, TX), Senju (Osaka, Japan), and Teika (Toyama, Japan) pharmaceutical companies, respectively.

PGE_2 was obtained from Funakoshi Chemicals (Tokyo, Japan). PGE_2 was dissolved in 100% ethanol and stored at −70°C. PGE_2 solution was diluted to 5% ethanol with 0.9% NaCl just before use. Epinephrine ophthalmic solution was diluted with 0.9% NaCl.

Topical Instillation of Antiglaucoma Agent or Placebo

In one eye, 50 μL 0.1% antiglaucoma agent or placebo (0.9% NaCl) was topically instilled. Instillation took place twice (60 and 30 minutes before PGE_2). We also performed single instillations to examine dose and time dependency. The bottles were masked, and the person who

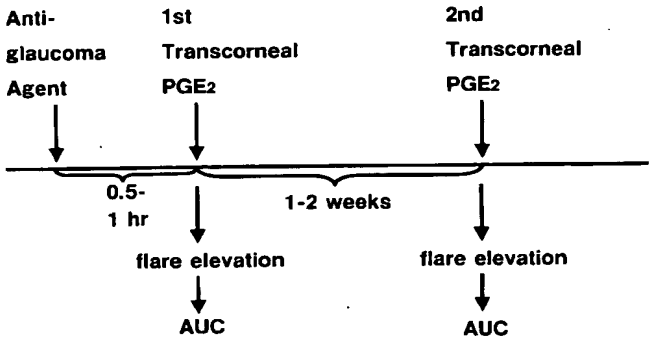

Figure 1. Two applications of PGE_2. The eyes received two transcorneal diffusions of PGE_2 at 1- or 2-week intervals.

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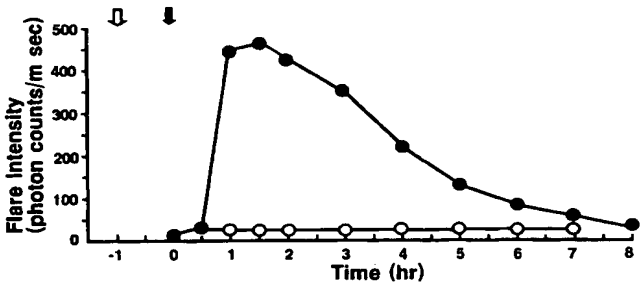

FIGURE 2. Changes in flare intensity after transcorneal diffusion of PGE_2, with and without topical instillation of apraclonidine. (●) Transcorneal application of PGE_2 (7.09 × 10-2 mM) for 4 minutes (solid *arrow*). (○) Apraclonidine (1.15%) topically instilled 60 minutes before PGE_2 application (*open arrow*). Data are the mean (n = 6).

administered the eye drops had no preliminary knowledge of the contents.

Transcorneal Diffusion of PGE_2

For transcorneal diffusion, a glass cylinder (11 mm in diameter) was attached to the cornea, as described by Hirata et al.[1] Next, 600 μL of PGE_2 solution (25 μg/mL or 7.09 × 10^{-2} mM) was delivered into the cylinder and pipetted out 4 minutes later. The cylinder was removed, and the corneal surface and conjunctival sac were rinsed with 20 mL 0.9% NaCl. The eyes received a second transcorneal application of PGE_2, 1 or 2 weeks later (Fig. 1). PGE_2-induced flare elevation was measured in the eye pretreated with antiglaucoma agent or placebo (0.9% NaCl) and again after the second PGE_2 application.

Aqueous Flare Measurement

Aqueous flare was measured with a laser flare cell meter (model FC 1000; Kowa, Tokyo, Japan), according to the method described by Sawa et al.[5] A laser flare-cell meter measured intracameral proteins. Five measurements were taken at each time point to obtain a mean value. The measurement was taken in the midportion of the anterior chamber. The sampling area was 0.075 mm^2.

Aqueous flare elevation was expressed as the area under the curve (AUC) for each eye. Inhibition was estimated from the AUCs in the same eye by the following equation: inhibition (%) = 1 − [(AUC with treatment)/(AUC without treatment)] ×100. The measurer had no preliminary knowledge of the treatment.

Statistics

Statistical analysis was performed using the Dunn multiple comparisons procedure. $P < 0.05$ was considered significant.

RESULTS

After topical instillation of epinephrine 1.25%, the iris became slightly pale. Other eye drops induced no change in iris color. Two instillations (60 and 30 minutes before PGE_2) of apraclonidine, epinephrine, dipivefrin, timolol, nipradilol, dorzolamide, and pilocarpine did not induce aqueous flare elevation.

No marked changes in the systemic condition, including body weight and behavior, were noted after the transcorneal diffusion of PGE_2 (7.09 × 10^{-2} mM).

After PGE_2 was administered, aqueous flare increased, reached its maximum (470 ± 37 photon counts/ms) at 60 to 90 minutes, and then gradually decreased and returned to baseline level after 7 to 8 hours (Fig. 2). When apraclonidine 1.15% was topically instilled 60 minutes before PGE_2, aqueous flare did not increase. Single instillation of apraclonidine 1.15%, two instillations of epinephrine 1.25%, two instillations of dipivefrin 0.1%, and two and one instillations of dipivefrin 0.04% eye drops inhibited 98%, 96%, 87%, 73%, and 47% of PGE_2-induced increase in aqueous flare, respectively (Table 1).

Timolol 0.5%, nipradilol 0.25%, dorzolamide 1%, and pilocarpine 2% eye drops had no effect on the increase in PGE_2-induced flare. The effect of a single instillation of epinephrine on the increase in PGE_2-induced aqueous flare in pigmented rabbits is shown in Table 2. Topical instillation of epinephrine inhibited flare elevation in a dose-dependent manner (0.2%–1.25%). Instillation of 1.25% epinephrine 30 minutes before PGE_2 application inhibited 90% of PGE_2-induced aqueous flare elevation.

DISCUSSION

We have reported that topical clonidine inhibits the increase in PGE_2-induced aqueous flare.[3] Topical apraclonidine (*p*-aminoclonidine) reduces the increase in intraocular pressure and aqueous humor protein after YAG and argon laser irradiation of the rabbit iris.[6,7] In the present study, topical apraclonidine also inhibited the PGE_2-induced elevation in aqueous flare in rabbits.

Several researchers have reported a relationship between epinephrine and aqueous humor: Townsend and Brubaker[8] postulated that epinephrine increases the rate of the uveoscle-

TABLE 1. Effects of Eye Drops on PGE_2-Induced Aqueous Flare Elevation in Pigmented Rabbits

Eye Drop	Concentration (%)	Number of Instillations*	Inhibition of Flare Elevation (%)	P
NaCl	0.9	2	1 ± 9	
Apraclonidine	1.15	2	98 ± 1	<0.01
		1	98 ± 2	<0.01
Epinephrine	1.25	2	96 ± 10	<0.01
Dipivefrin	0.1	2	87 ± 8	<0.01
	0.04	2	73 ± 6	<0.01
		1	47 ± 9	<0.01
Timolol	0.5	2	9 ± 10	>0.05
Nipradilol	0.25	2	0 ± 7	>0.05
Dorzolamide	1.0	2	4 ± 8	>0.05
Pilocarpine	2.0	2	8 ± 7	>0.05

n = 6 eyes.
* One, 60 minutes before PGE_2, two, 60 and 30 minutes before PGE_2.

TABLE 2. Effects of a Single Instillation of Epinephrine on PGE_2-Induced Aqueous Flare Elevation in Pigmented Rabbits

Eye Drop	Concentration (%)	Instillation before PGE_2 (min)	No. of Eyes	Inhibition of Flare Elevation (%)	P	
NaCl	0.9	30	6	2 ± 10	<0.01	
Epinephrine*	0.21	30	6	30 ± 9	<0.05	
	0.42	30	6	58 ± 9	<0.05	<0.05
	1.25	30	5	90 ± 10		
	0.42	60	4	36 ± 8		<0.05
	0.42	120	4	23 ± 9		<0.01
	0.42	180	4	0 ± 8		

* Ophthalmic solution was diluted with 0.9% NaCl.

ral outflow pathway in humans. Camras et al.[9] reported on the inhibition of epinephrine-induced reduction of intraocular pressure by systemic indomethacin in humans. Miyake et al.[10,11] reported that epinephrine induces disruption of the blood-aqueous barrier several months after drug administration in rabbits and humans. Anderson and Wilson[12] described inhibition by indomethacin of the increased facility of outflow induced by adrenaline in rabbits. Mori et al.[13] reported that a single instillation of epinephrine affects neither the protein concentration in the anterior chamber nor the aqueous flow rate in humans. In our present study, topical instillation of epinephrine and dipivefrin (dipivalyl epinephrine) inhibited the PGE_2-induced elevation of aqueous flare in rabbits. Miyake et al.[14] further reported synthesis of PGE_2 in rabbit eyes with topically applied epinephrine.

However, our results showed that a single instillation of 1.25% epinephrine 30 minutes before PGE_2 inhibited 90% of the increase in PGE_2-induced flare. The difference between our results and the findings reported by Miyake et al.[10,14] may be due to the varied instillation times. Okada and Shimada[15] reported that intravenous epinephrine and intramuscular steroid inhibit the increase of permeability of the blood-aqueous barrier induced by reverse passive Arthus reactions in rabbits. Our results were similar to those described by Okada and Shimada.[15] The iris in our animals became slightly pale after topical instillation of 1.25% epinephrine. Vasoconstriction induced by epinephrine may play a role in inhibition of the PGE_2-induced elevation in aqueous flare. Topical epinephrine or dipivefrin effects on the elevation of aqueous flare after argon laser iridotomy should be examined in humans.

We have reported that betaxolol inhibits the PGE_2-induced elevation in aqueous flare in rabbits and suggest that the calcium-channel blocking activity of betaxolol may be involved in the inhibition.[4] Miichi and Nagataki[16] reported that timolol does not alter the function of the blood-aqueous barrier in the cynomolgus monkey. Kanno et al.[17] reported that a single instillation of nipradilol shows no significant effect on blood-aqueous barrier permeability in rabbits.

In our present study, timolol and nipradilol did not inhibit elevation of aqueous flare. It is unlikely that the β-blocking activity of the drugs is involved in the inhibition of the elevation of flare in rabbits. Mori and Araie[18] reported that timolol induces elevation of protein concentration in humans. The discrepancy between our results and the findings reported by Mori and Araie[18] may be due to the difference in species.[19]

In the present study, topical dorzolamide and pilocarpine did not alter the PGE_2-induced elevation of aqueous flare. Miichi and Nagataki[16] reported that the blood-aqueous barrier was not altered by pilocarpine in the cynomolgus monkey. Our findings support this.[16]

PGE_2-like activity was detected in the aqueous humor after paracentesis in rabbits,[20] and it may be involved in traumatic iridocyclitis in rabbits. The blood-aqueous barrier in rabbits has a unique sensitivity to PGs.[19] Therefore, the findings in the present study are not representative of the effects seen in humans. The mechanisms of inhibition by epinephrine and dipivefrin of the PGE_2-induced elevation of aqueous flare in rabbits should be investigated.

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